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RESEARCH ARTICLE

Marine Algal extract as a biostimulant to improve tolerance to salinity in lettuce plants

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ABSTRACT

The purpose of this study was to investigate the impact of marine algal extract as foliar spray on growth, physiological, mineral composition, different osmoprotectant levels, various parts of antioxidant system, and gene expression of greenhouse lettuce (*Lactuca sativa* L.) plants grown under saline conditions (50mM and 100mM). Through LC/MS analysis, the algal extract's phenolic composition was determined by its abundance in quinic acid, gallic acid, kaempferol and luteolin. The concentration of leaf proline increased while the biomass production, chlorophyll fluorescence, SPAD index and mineral composition all decreased as a result of salt stress. The salt-stress tolerance mechanism in lettuce involved the expression of genes that encode antioxidant enzymes, including *LsSOD, LsCAT*, and *LsAPX*, in both stressed and control plants, which demonstrates their important roles. The findings revealed that plants that were treated with marine algal extract had a greater ability to handle salt challenge, as evidenced by a significant ($p < 0.05$) increase in plant growth, SPAD index, Fv/Fm ratio, a better nutritional status in addinto a better osmoprotection against salt stress. Malondialdehyde level, Hydrogen peroxide and stability index levels were significantly decreased. Furthermore, the antioxidant system saw a significant improvement, as demonstrated by the increased activity of catalase (CAT), superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) under moderate stress conditions (50mM) and more abundant *LsCAT* transcripts in the stressed plants. Overall, our findings indicate that marine algal extract appears to be an effective biostimulant product for treating lettuce under saline conditions.

Keywords: Antioxidant enzymes; Chlorophyll fluorescence; Gene expression; Growth performance; *Lactuca sativa* L., Marine algal extract; Mineral composition; Osmoprotectants

INTRODUCTION

Salt is a severe environmental stress that affects virtually all crops by decreasing growth components during the entire growth cycle (Negrao et al., 2017). It leads to significant reductions in agricultural production worldwide. (Hernández, 2019).

Ionic and osmotic stress caused by sodium accumulation results in retarded growth in terms of biomass production, decreased chlorophyll content, and hinders the consumption of minerals (Ahmad et al., 2014). Long-term salinity exposure can lead to specific ion toxicity, hormonal and nutritional imbalance, and decreased water potential (Siddiqui and Khan, 2013; Buedo and Gonzalez, 2020). This result in secondary stress, specifically oxidative stress, due to the excessive generation of reactive oxygen species (Munns and Tester, 2008). The plants' physiology is impacted by salinity stress, which decreases their photosynthetic rate by multiple means, including a reduction in carbon dioxide supply due to stomatal closure. The efficiency of photosynthesis decreases due to non-stomatic limitations caused by salinity, which affects photosynthesis by reducing pigment content and the flow of electrons through the chloroplast. Furthermore, this results in a decrease in the cell membrane carbon dioxide permeability, an increase in senescence, and a change in enzymatic activity (Kafi and Rahimi, 2011).

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The increase in ROS is partially balanced by antioxidant enzymes that scavenge it (Bose et al., 2014). Cell damage caused by oxidative stress is largely prevented by these enzymes (Gupta and Huang, 2014). The cell experiences lipid peroxidation and excess production of MDA, a product of membrane peroxidation, as a result of the increase in free radicals (Gharibi et al., 2016).

Lettuce is a highly recommended healthy food source with positive organoleptic properties and abundant bioactive compounds which have made it an important part of the human diet (Kim et al., 2016). In the Mediterranean region, saline water is frequently utilized for irrigation in this species, which is considered one of the biggest vegetable crops (Lucini et al., 2015).

Starting with a soil electrical conductivity of around 2 $dS \cdot m^{-1}$ and water electrical conductivity of around 0.9 $dS.m^{-1}$, the growth of lettuce plants is hampered (Machado and Serralheiro, 2017). Thus, the expansion of studies on the lettuce salt stress tolerance is crucial.

Although plants can generate various types of antioxidants that can alleviate abiotic stress impacts, exogenous applications need to be investigated (Garcia-Mina and Hadavi, 2016).

The effect of biostimulants on plant growth and productivity has been analyzed by several studies through short-term experiments. Numerous reports have documented the successful use of these biostimulants, whether used individually or in mixtures, to prevent abiotic stresses. It appears that natural plant extracts can be used as an effective tool for enhancing plant growth and stress tolerance by regulating multiple physiological processes (Desoky et al., 2020).

Beneficial microorganisms were reported to have similar results. Introducing beneficial microorganisms into crop production systems is regarded as a sustainable strategy to guarantee competitive yields across multiple crops and enhance resource efficiency (Singh et al., 2023; Kour et al., 2020).

Salt tolerance has been reported to be improved by plant-derived protein hydrolysates in numerous studies. Under abiotic stresses, plants have benefited from the use of these biostimulants by accumulating bioactive compounds that minimize the extent of plant productivity reduction due to salinity (Colla et al., 2017; Malécange et al., 2023).

Marine Algal extracts are included in the category of biostimulants and are utilized to improve plant tolerance. Different molecular and physiological mechanisms can be activated by algal extracts to enhance plant stress tolerance (Carillo et al., 2020). Specifically, the antioxidant response can be boosted and photosynthetic process, growth and yield can be enhanced by the activation of specific metabolic pathways in these extracts. (Dell'Aversana et al., 2021;Samuels et al., 2022).

These studies show the capability of exogenous products to positively regulate multiple physiological processes. Therefore, investigating the incredible biostimulant potential to enhance the production of bioactive molecules, supporting the antioxidant activity and osmoregulation mechanism under salt stress conditions, would be a successful strategy for a sustainable agriculture.

The use of Marine Algal Extracts is environmentally friendly, due to their biodegradability, non-toxicity, non-pollution, and lack of hazards for humans and ecosystem (Khan et al., 2009). A variety of substances are present in them that promote growth. Besides boosting growth, there is increasing evidence that Seaweed Extracts can improve plant stress tolerance (Khan et al., 2009; Sharma et al., 2013).

Despite these advantages of Marine algal Extracts for certain plant species under salt stress, the findings concerning physiological and biochemical mechanisms are still poorly understood. The primary photosynthetic, enzymatic, and molecular processes associated with salinity tolerance in vegetables such as lettuce need to be clarified by studies.

Considering the critical role of Marine algal extracts in plant stress responses and management, the purpose of this study was to test the effect of marine algal extract on the tolerance of lettuce plants to salinity conditions.

The purpose of the current work was to evaluate the effects of exogenous *Ulva lactuca* extract on mitigating adverse effects on growth traits and physiological characteristics of lettuce plants caused by high salinity. In addition, we examined the potential of algal extract application on the essential osmolytes accumulation, the integrity of leaf tissue, antioxidant enzyme activities and gene expression in lettuce plants under salt stress conditions.

MATERIAL AND METHODS

Seaweed collection and biostimulant preparation

A macroalgae species *Ulva lactuca* was used to prepare the marine algal extracts. The algae were harvested in February 2021 at the sea of Carthage, in the Northeast of Tunisia. The seaweed material was cut into small pieces and stored at -20 °C until use. After boiling one kilogram of seaweed in 1L of distilled water for 60 minutes, it was filtered through a double-layered muslin cloth to remove any impurities (Sivasankari et al., 2005). The seaweed concentration was determined by selecting these filtrates as 100% (Fig. 1).

The algal extract concentrations were obtained through the incorporation of distilled water.

Seaweeds material analysis

Phenol extraction

Using the method described by Ruiz-Ruiz et al. (2016), 5 grams of seaweed material and 10% distilled water were grinded to produce aqueous extracts.

Phenolic compound analysis

Phenolic compounds were examined using High-Performance Liquid Chromatography-Mass Spectrometry (HPLC-MS). An injection of 10 µl was made into the column (C18□ 5; 250 mm × 4 mm) and the flow rate was maintained at 0.4 ml/min at 40 °C at the following gradient conditions for the mobile phase include acetonitrile/0.25% formic acid (F) and water (W): F: W (10:90) for 5 minutes, changed to F: W (50:50) for 30 minutes and held for 5 minutes, changed to F: W (10:90) for 5 minutes. By comparing standard phytochemicals, HPLC-MS system analysis was used to identify phenolic compounds.

Plant materials, growth conditions and treatments

The experiment was conducted in a greenhouse situated on the experimental station of the Higher Agronomic Institute of Chott Mariem, Tunisia. The soil samples were mixed with peat (4:5 ratio). The physicochemical analysis of the experimental soil was performed before seeding and the principal soil properties are presented in Table 1.

Three NaCl concentrations (0 mM, 50 mM, or 100 mM) were selected in conjunction with biostimulant applications (Control, Seaweed Extract).

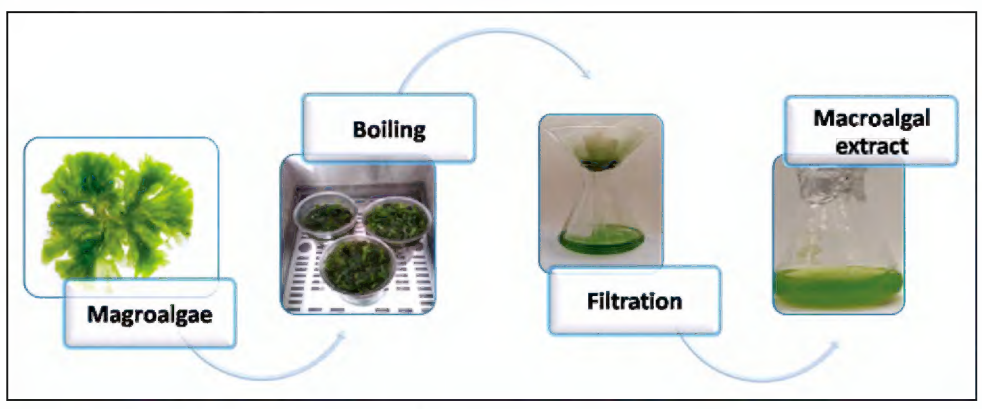

Fig 1. Marine Algal Extract Preparation.

Table 1: Physical and chemical characteristics of the soil used in the experiment

Soil characteristics	Value
Textural class	sandy loam
pH	7.06
EC (µs cm^{-1})	910
CEC ($cmol.kg^{-1}$)	8.03
Organic matter (%)	1.21
Available nutrient (mg kg^{-1} soil)	
N	159
P	29
K	122

A total of 6 treatment combinations and 18 experimental units were achieved by arranging the treatments into a 3 × 2 factorial in a completely randomized design with three replications. Hogland nutrient solution was utilized as a base nutrient source across the pots. Five-week-old seedlings were moved to plastic pots. Saline treatment was initiated 20 days after transplantation.

Electrical conductivity levels in the irrigation water were 0.02, 4.13, and 10.1 dSm^{-1}. The control treatment was watered with distilled water (pH 6.2; EC = 0.02 dS/m). The application of sodium chloride was gradually increased to 25 mM per day to prevent salt shock.

At seven-day intervals during the growing season, plants were treated with seaweed extract using a 50% foliar spray (2 mL per plant), starting from six days after transplantation. Table 2 includes all treatment combinations.

Evaluation of plant growth parameters

Vegetative growth was estimated based on the leaf area, fresh weight and dry weight. After 38 days of transplantation, lettuce plants were harvested. Images were processed using ImageJ software to measure leaf areas by taking photographs of well-spread leaves (National Institutes of Health, Bethesda, MD, USA).

Mineral analysis

Samples of leaves and roots were taken 40 days after transplantation, then crushed using a mortar using a mortar and filtered; Sodium (Na^+) and Potassium (K^+) were recorded from the filtrate using ion concentration meters (Cardy Potassium K^+ Meter) and (Cardy Sodium Na^+ Meter). The measurements were carried out on 3 repetitions per treatment.

Evaluation of Photosynthetic parameters

SPAD Index measurements

Three nondestructive measurements of the chlorophyll content were determined by the Soil Plant Analysis Development (SPAD index) at 10, 20, and 35 days after transplantation. To measure foliar chlorophyll concentration

Table 2: Experimental treatments applied to lettuce seedlings

T1	Control: plants irrigated by the basic nutrient solution.
T2	Plants irrigated with the basic nutrient solution with 50 mM NaCl,
T3	Plants irrigated with the basic nutrient solution with100 mM NaCl
T4	Plants treated with the Marine algal Extract and irrigated with the basic nutrient solution.
T5	Plants treated with the Marine algal Extract and irrigated by the basic nutrient solution with 50 mM NaCl,
T6	Plants treated with the Marine algal Extract and irrigated by the basic nutrient solution with 100 mM NaCl.

in a rational unit, a portable chlorophyll SPAD-502 instrument was employed. Each replicate included ten fully expanded leaves from 3 plants for each treatment.

Maximum quantum use efficiency of psii measurements

On the same dates, a chlorophyll fluorometer (Opti-sciences model) was used to assess Chlorophyll fluorescence, measuring Fv/Fm (Maximum quantum efficiency of PSII photochemistry) after dark acclimation for 20 minutes. The maximum quantum yield of PSII chemistry can be determined reliably by Fv/Fm, as demonstrated both theoretically and empirically (Bulter, 1978). Unstressed leaves have a high consistency value of Fv/Fm that correlates with the maximum quantum yield of photosynthesis at ~ 0.83 (Demmig and Björkman, 1987).

Leafy tissue integrity assessment

Relative water content (RWC)

The Relative Water Content of lettuce leaves was measured using the method proposed by Barrs and Weatherleyt (1962). The RWC was calculated through the formula:

$$Relative Water\ Content\,(\%) = \frac{Fresh\ weight - Dry\ weight}{Turgid\ weight - Dry\ weight}$$

Proline content

Proline content was analyzed using the method of Monneveux and Nemmar (1986). L-proline was used to perform calibrations.

Soluble sugars content

Soluble sugars content was measured using phenol sulfuric acid according to Dubois et al. (1956). Soluble sugars content was expressed as µg g^{-1} DW.

Lipid peroxidation

To evaluate lipid peroxidation, the content of malondialdehyde (MDA) was measured by the thiobarbituric acid (TBA) reaction (Hernández and Almansa, 2002). The amount of MDA was determined by utilizing an extinction coefficient of 155 mM^{-1} cm^{-1} and reported as µmol g^{-1} FW.

Hydrogen peroxide (H_2O_2) content

The protocol of Velikova et al. (2000) was used to measure the concentration of H_2O_2. A standard calibration curve was used to determine the H_2O_2 level and it was expressed in µmol g-1 FW.

Leaf electrolyte leakage (EL) level

According to Kaya et al. (2002), membrane permeability was measured by electrolyte leakage. Two plants were used to collect lettuce leaf samples and divide them into 1 cm segments for every replication. The samples were floated in flasks with distilled water and incubated) on a shaker (100 rpm) at room temperature (25°C for 24 h. After incubation, the initial electrical conductivity of the solution (EC1) was observed. The same samples were then heated for 20 minutes at 120°C in an autoclave and a second reading electrical conductivity (EC2) was measured after cooling the solution at room temperature. *EC1/EC2* was used to calculate the electrolyte leakage and expressed it as a percentage.

Antioxidant enzymes assays

Enzyme extraction

The modified protocol of Rubio et al. (2002) was followed for the extraction of enzymes. After being ground in liquid nitrogen, the frozen plant samples (0. 5 g) were extracted with 50 mM phosphate buffer (pH 7.8) that contained 0.1 mM EDTA, 1 mM PMSF, 10 mM DTT and 1% insoluble PVPP. For the measurement of ascorbate peroxyoxidase (APX) activity, 5 mM ascorbate was included to the extraction buffer. Centrifugation was conducted (13,000 g, 20 min, 4°C) on the homogenates obtained, and the supernatants resulting from this centrifugation will be tested for enzymes. The total activity of the multi-enzymatic system was measured with enzyme activities tested and expressed in units per mg protein. The Bradford method (1976) was used to determine the protein concentration of the samples, with bovine serum albumin as the standard.

Antioxidant enzyme activities

The protocol developed by Nakano and Asada (1981) was utilized to determine ascorbate peroxidase (APX) activity. The expression for peroxidase activity was in terms of µmol H_2O_2 min^{-1} mg^{-1} protein.

The evaluation of catalase activity was carried out by measuring the decrease in absorbance (240 nm, 1 minute) that resulted from the degradation of hydrogen peroxide (H_2O_2) (Claiborne, 1984). The extinction coefficient of 36 mM^{-1} cm^{-1} was used to determine catalase activity and expressed in µmol H_2O_2 min^{-1} mg^{-1} protein.

Using the Beyer and Fridovich (1987) method, superoxide dismutase (SOD) activity was recorded by photoreducing Nitrotetrazolium Blue Chloride (NBT). The amount of enzyme that produces 50% inhibition of NBT reduction is defined as one unit of SOD. The activity of superoxide dismutase has been reported in Units SOD min^{-1} mg^{-1} protein.

Gene expression

RNA isolation and synthesis of cDNA

Lettuce leaf samples were collected after two applications of the biostimulants and one application of the Nacl

concentrations. The protocol described by Chang et al. (1993) was followed for extraction of total RNA. The NanoDrop Spectrophotometer and an electrophoresis gel were used to assess RNA quantity and quality. Based on the manufacturers' instructions, a First Strand cDNA Synthesis Kit was utilized to generate the first-strand cDNAs.

Real-time reverse transcription-PCR

The primer sequences *for LsSOD, LsCAT and LsAPX* genes are presented in Table 2. The samples were standardized using a control gene (UBQ). The 7300 Real-Time PCR System was used for RT-qPCR, and the Maxima SYBR Green/ROX qPCR Master Mix (2X) kit (Biomatik) was used as the method to perform the analysis. Each reaction mix (20 µL) included 1 µL of the primer at 10 µM, 10 µL Maxima SYBR Green/ROX qPCR Master Mix (2X), 6 µL dd H_2O and 2 µL cDNA (50 ng). For every sample, the reactions were done in triplicate. The conditions of Thermocycler were 95°C for 5 min, followed by 40 cycles at 95°C for 30 s and 60°C for 1 min. After the final PCR cycle, a melt curve analysis was conducted to verify the specificity of the PCR amplification at 55°C to 94°C. The $2^{-\Delta\Delta CT}$ method was employed to analyze the relative expression levels in accordance with Schmittgen and Livak (2008) description.

Statistical analysis

All experiments were executed three times and the data was presented in terms of mean values and standard deviations. The data was analyzed statistically using SPSS (version 11.0) software (SPSS Inc., Chicago, Illinois, USA). One-way analysis of variance (ANOVA) and Tukey's honestly significant difference (HSD) test ($p < 0.05$) were used to examine the statistical significance of different treatments. The 'corrplot' package was used to perform correlation matrix visualization in R statistical programming environment (Version 4.0.3).

RESULTS

Characteristics of algal extract

Table 4 summarizes the total flavonoids, total phenols, and amounts of individual phenolic compounds from Marine Algal extract. Eight phenolic compounds were identified in the Marine Algal extract including three phenolic acids (quinic acid, gallic acid and 1,3-di-O-caffeoquinic acid) and five flavonoids (Kampherol, Rutin, Apegenin, Luteolin and Acacetin). Total amount of polyphenols present 1.418 mg/g. Flavonoids represented the major class of phenols in the extract (1.024 mg/g), while phenolic acids were poorly represented with a percentage of 27.78% of total phenols (0.394 mg/g). The major phenolic compound is kampherol.

Table 3: Primer sequences utilised for real-time quantitative PCR (q-PCR) investigation of LsSOD, LsAPx, LsCAT and LsUBQ

Transcript	Forward and reverse primer
LsSODF	CGGTCCAACAACTGTCAATG
LsSODR	CAGGAGCACCATGTGTATGC
LsCATF	CCTGGTGTCCAAACTCCTGT
LsCATR	ATTGTTCCCCACCAAATCAA
LsAPXF	ATGCAGTGTCCCACATGAAA
LsAPXR	ATCAGGTCACCCCTCTCCTT
LsUBQ	AAGACCTACACCAAGCCCAA
LsUBQ	AAGTGAGCCCACACTTA C

Table 4: Phenolic compounds concentration (mg/g) in the marine algae extractusing LC/MS analysis

Phenolics	Retention time	Amount (mg/g)
Quinic acid	1.621	0.272
1,3-di-O-caffeoyquinic acid	1.630	0.049
Gallic acid	1.627	0.073
Rutin	21.694	0.047
kaempferol	29.323	0.872
Apegenin	32.143	0.022
Luteolin	32.445	0.078
Acacetin	38.098	0.005
Total phenolic content (mg GAE. g^{-1})		1.974
Total flavonoid content (mg CE .g^{-1})		12.15

Characteristics of lettuce plants

Growth parameters

In this study, lettuce plants exposed to salt stress experienced a decline in the growth traits. Compared to the control plants, plant fresh and dry weight were reduced by 58.78% and 48.66%, respectively, due to Nacl stress exposure (Table 5). Likewise, Leaf area per plant was negatively affected by salt stress treatment, and it was lower by 32.20% compared to the unstressed plants.

Under severe stress conditions (100 mM NaCl), seaweed extract application resulted in a 30.44 % and 36.04% rise in dry and fresh weight, respectively, compared to the untreated plants lettuce (Table 5).Seaweed extract application resulted in a 10.88% increase in leaf area (Table 5).

Mineral analysis

The concentrations of sodium (Na^+) and potassium (K^+) depending on NaCl level and biostimulant application are depicted in Fig. 3.

Under saline conditions, significant increase in Na^+ concentration was observed in all lettuce plants' growth. The greatest level of Na^+ was detected in plants untreated by biostimulants at 100 mm NaCl.

Similarly, Fig. 1 displays the decrease in K^+ in shoots due to salt stress.

Table 5: Effect of applying the biostimulant on the development characteristics of lettuce plants cultivated at three different salinity concentrations at 38 days after transplantation

Treatments		Shoot Fresh Weight (g plant^{-1})	Leaf Area per plant (cm^2)	Shoot Dry Weight (g plant^{-1})
Biostimulant (B)	**Salinity (S)**			
Standard solution without biostimulant	0 mMol Nacl	104.26±9.22^{a}	118.36±3.76^{a}	6.69±0.46^{ab}
	50 mMol Nacl	80.33±6.01^{bc}	106.33±4.96^{c}	5.05±0.90^{c}
	100mMol Nacl	65.66±1.72^{c}	89.53±6.43^{c}	4.50±0.24^{c}
Standard solution+Biostimuat	0 mMol Nacl	118.80±15.12^{a}	144.16±6.38^{b}	7.86±0.40^{a}
	50 mMol Nacl	109.73±5.62^{a}	114.93±9.90^{c}	7.48±0.07^{ab}
	100mMol Nacl	102.66±4.02^{ab}	100.46±1.27^{c}	6.47±0.46^{b}
Significance				
S		ns	*	ns
B		*	ns	*
S X B		ns	*	ns

Values are mean of three replicates±standard error. Mean values in a column with different letters are significantly different at P<0.05 (Duncan's multiple-range test).Significance levels are represented by P>0.05, ns, not significant, * significant at P≤0.05, ** significant at P≤0.01. S: Salinity, B: Biostimulant application

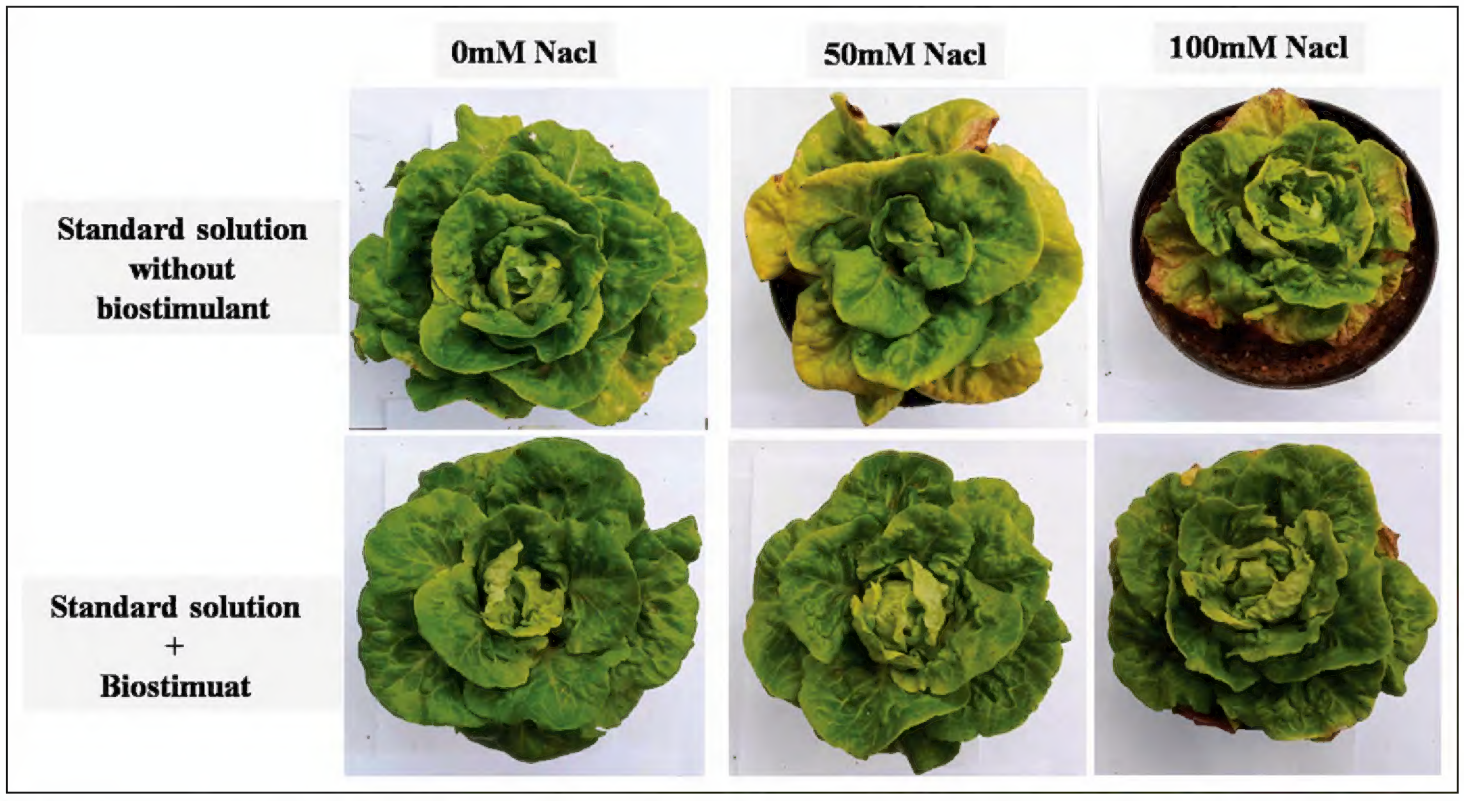

Fig 2. Influence of applying algae extract on phenotypic characteristics of lettuce plant under control conditions (0 mM NaCl), 50 mM, and 100 mM salt stress.

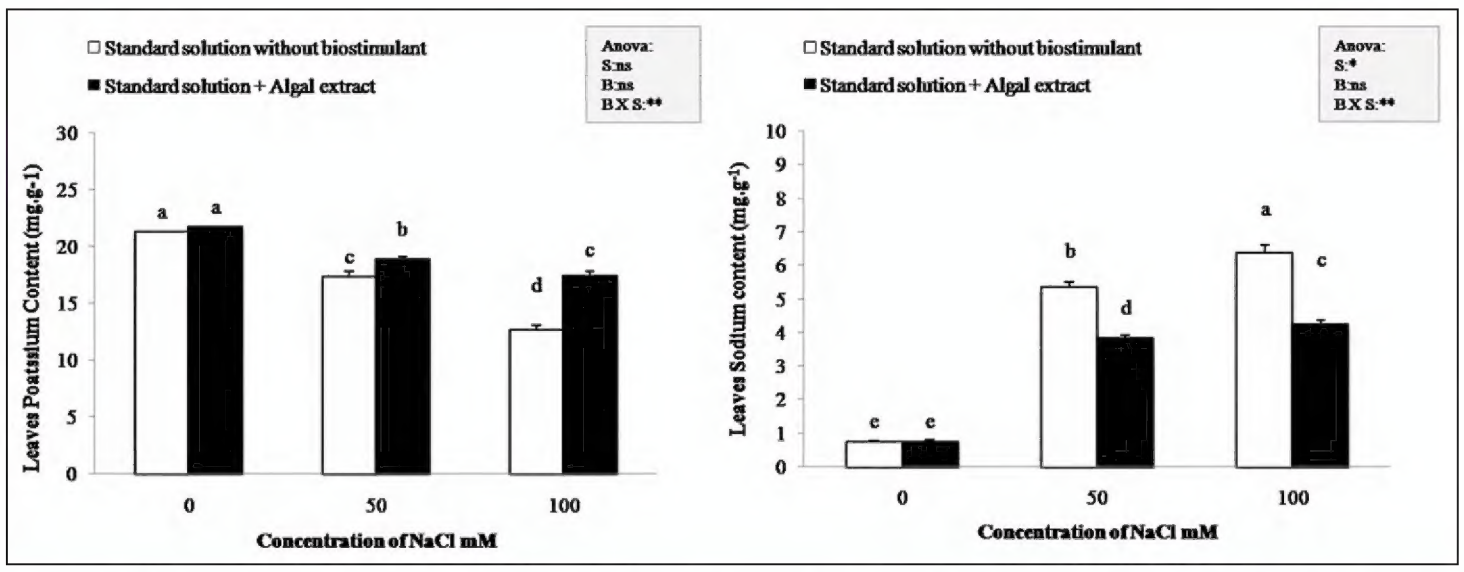

Fig 3. Effects of Algal Extract application on Potassium and Sodium in lettuce plants cultivated at three Nacl levels at five weeks after transplantation. Values are mean of three replicates, and error bars represent ±SD. The bars surmounted with the same letter are significantly comparable (Duncan's multiple-range test) ns, not significant, * significant at P ≤ 0.05, ** significant at P ≤ 0.01. S: Salinity, B: Biostimulant application.

Biostimulants typically reduce the concentration of Sodium (Na^+) and increase the concentration of Potassium (K^+) under saline conditions. The accumulation of Na^+ in leaves of plants grown under 100 mM NaCl was clearly influenced by seaweed extract.

Photosynthetic efficiency

Regardless of the biostimulant treatment, the SPAD index was significantly decreased by progressive increase of the NaCl concentration at 10 and 20 DAT. At 0 mM NaCl, plants treated with seaweed extract had the highest SPAD values (Table 6).

Table 6: Effect of applying the biostimulant on physiological parameters of lettuce plants cultivated at three different salinity concentrations at 10, 20 and 35 days after transplantation

Treatment		Fv/Fm (10DAT)	Fv/Fm (20DAT)	Fv/Fm (35DAT)	Spad Index (10DAT)	Spad Index (20DAT)	Spad Index (35DAT)
Biostimulant (B)	**Salinity (S)**						
Standard solution without biostimulant	0 mMol Nacl	0,8±0,02^{ab}	0,83±0,01^{ab}	0,86±0^{a}b	31,33±0,025^{e}	34,23±0,23^{d}	32,25 ±0,29^{b}
	50 mMol Nacl	0,74±cd	0,81±0^{c}	0,79±0,01^{c}	30,36±0,33^{f}	32,13±0,1^{e}	31,39±0,39^{c}
	100mMol Nacl	0,72±0,01^{d}	0,79±0^{d}	0,79±0,01^{c}	27,29±0,35^{g}	29,23±0,23^{g}	24,35±0,38^{e}
Standard solution+Biostimuat	0 mMol Nacl	0,81±0^{a}	0,85±0^{a}	0,87±0,01^{a}	35,13±0,42^{b}	37,14±0,16^{g}	34,15±0,15^{a}
	50 mMol Nacl	0,79±0,02^{ab}	0,83±0^{b}	0,83±0,02^{b}	33,34±0,47^{d}	35,25±0,44^{c}	34,26±0,35^{a}
	100mMol Nacl	0,76±0.004^{bc}	0,81±0.01^{c}	0,83±0,02^{b}	31,23±0,29^{e}	30,05±0,09^{f}	29,9±0,44^{d}
Significance							
S		ns	*	ns	*	*	ns
B		ns	*	ns	**	ns	ns
S*B		*	ns	*	*	**	**

Values are mean of three replicates±standard error. Mean values in a column with different letters are significantly different at P<0.05 (Duncan's multiple-range test).Significance levels are represented by P>0.05, ns, not significant, * significant at P≤0.05, ** significant at P≤0.01. S: Salinity, B: Biostimulant application

At 10, 20, and 35 DAT, plants treated with NaCl experienced a decrease in the Fv/Fm ratio, irrespective of the biostimulant treatment (Table 6). Foliar spraying with seaweed extract improved the photosynthesis efficiency parameters of plants, regardless of the NaCl concentration. Treated plants with seaweed extract showed the highest values of Fv/Fm at 10, 20 and 35 DAT, compared to untreated lettuce plants.

Relative water content

An increase in salt stress from 0-100 mM caused a reduction in the relative water content of lettuce plants (Fig. 4). The RWC of treated plants was significantly higher than untreated plants, indicating that the adverse effects of salinity were mitigated. Despite the salt level, the seaweed extract treatment maintained higher water content than the untreated control ones. The highest RWC (85.36%) was found in non-stressed plants treated by seaweed extract, and the lowest RWC (63.10 %) was found in untreated plants at 100 mM salt application.

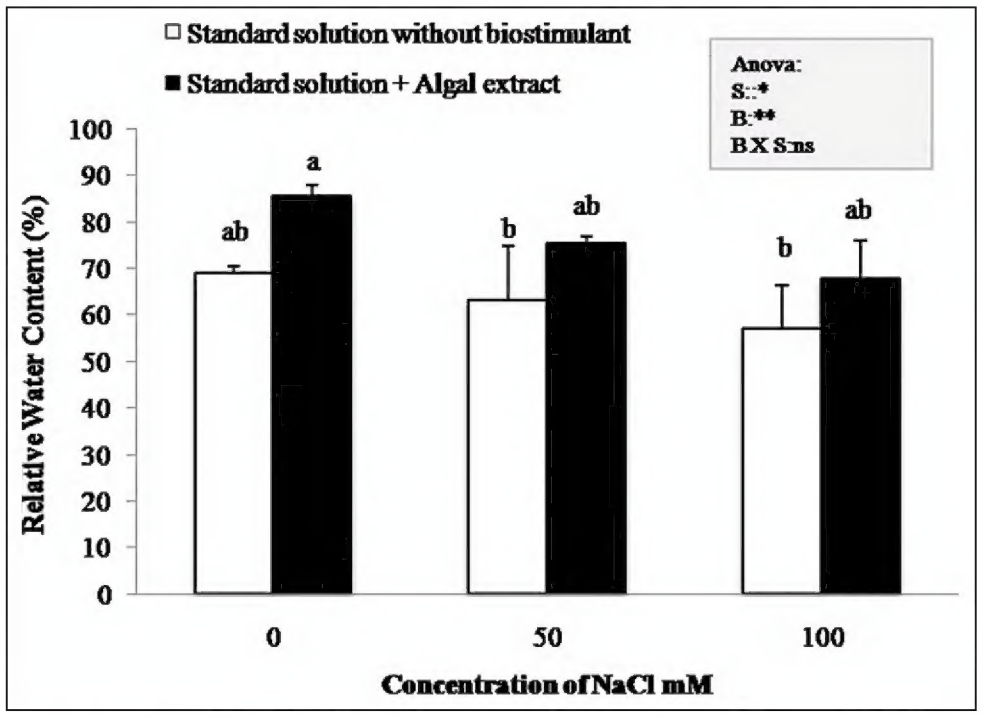

Fig 4. Effects of Algae Extract application on the relative water content (RWC) of lettuce plants cultivated at three different salinity concentrations. Values are mean of three replicates, and error bars represent ±SD. The bars surmounted with the same letter are significantly comparable (Duncan's multiple-range test) ns, not significant, * significant at P ≤ 0.05, ** significant at P ≤ 0.01. S: Salinity, B: Biostimulant application.

Proline content

In untreated plants by biostimulant, The three Nacl treatments did not differ statistically and the proline content was slightly elevated by salt stress. The standard nutrient solution and biostimulant treatments showed significant differences in proline analysis in leaves (Fig. 5). In plants treated with NaCl, the content of leaf proline was significantly elevated by 14.73% (Fig. 5). Exposure to 100 mM NaCl and seaweed extract increased the proline level by 22.42% compared to untreated lettuce.

Total soluble sugar content

At 50 mM NaCl (moderate stress conditions), total soluble sugars content was enhanced by 9.73%, in the untreated plants by seaweed extract (Fig. 6).

The soluble sugar content was significantly increased by the application of biostimulant. At 0 mM NaCl, the plants treated with the biostimulant had the lowest content. (93.53 µg.mg^{-1}DW) Compared to the controls, the content increased by 45.70% at 50 mM NaCl and by 42.54% at 100 mM NaCl.

Levels of oxidative stress indicator and membrane damages

The highest level of H_2O_2 was recorded by salinity at 100 mM NaCl (Fig. 7A). Despite the untreated controls having higher values, the H_2O_2 content decreased significantly when plants were biostimulated.

Similar to H_2O_2, salt stress caused an increase in MDA, comparable to the control. MDA values were decreased after seaweed extract application (Fig. 7B). On the other hand, the biostimulant application led to a remarkable decrease in the content of this product in lettuce.

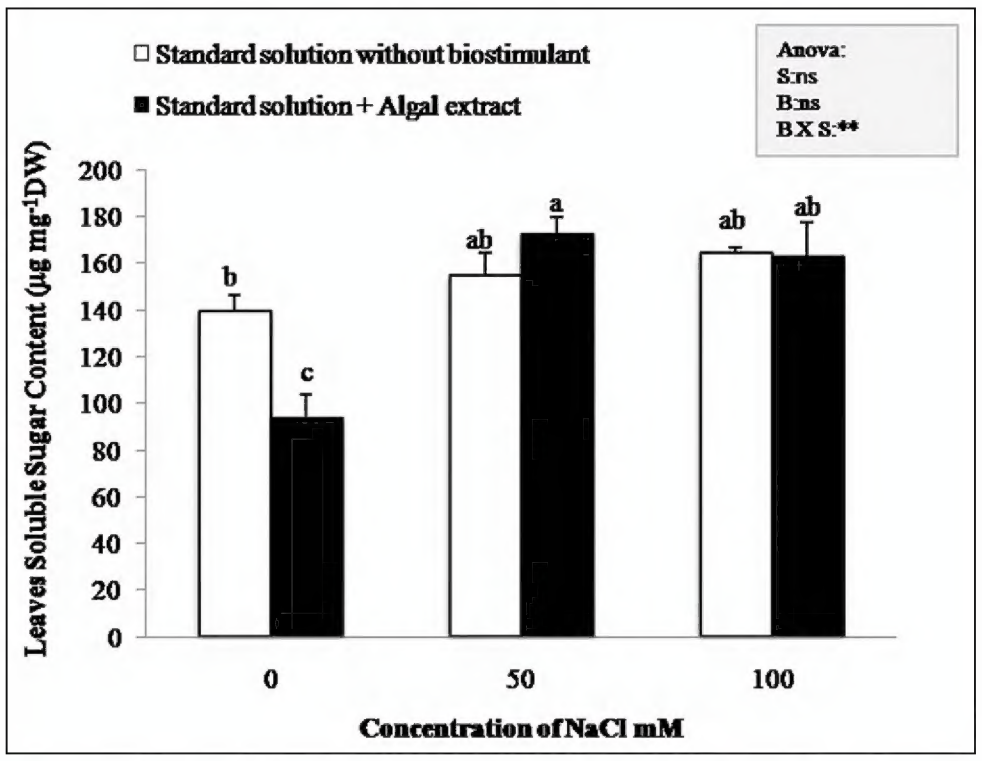

Fig 5. Effects of Algal Extract application on leaf proline content of lettuce plants cultivated at three different salinity concentrations. Values are mean of three replicates, and error bars represent ±SD. The bars surmounted with the same letter are significantly comparable (Duncan's multiple-range test) ns, not significant, * significant at $P \leq 0.05$, ** significant at $P \leq 0.01$. S: Salinity, B: Biostimulant application.

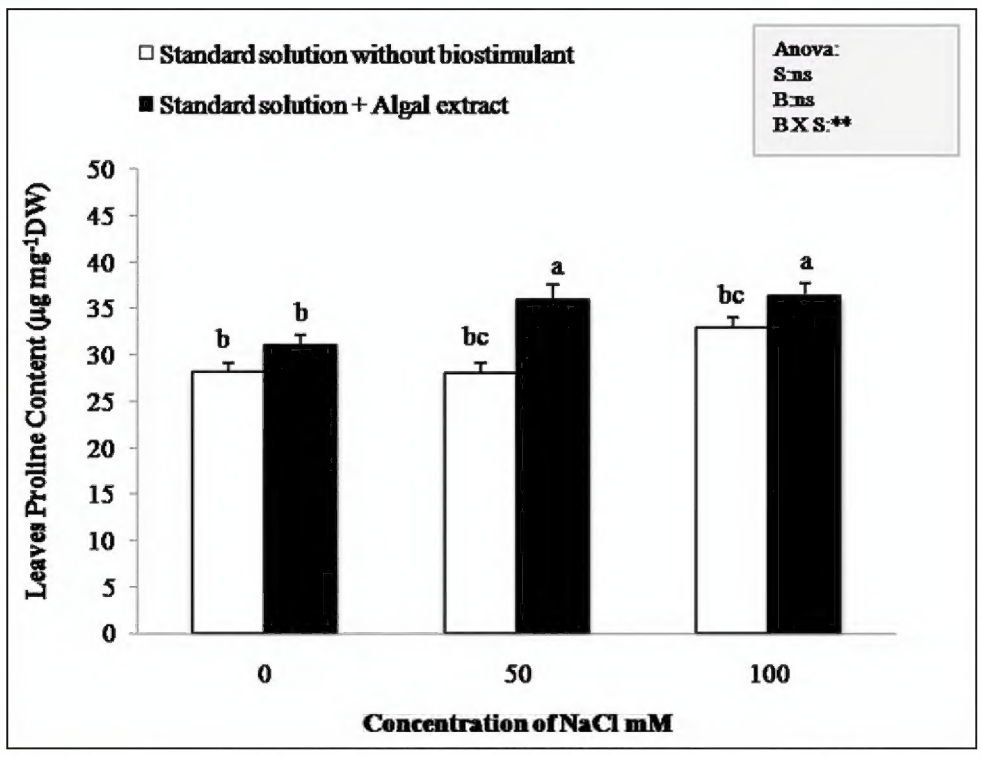

Fig 6. Effects of Algal Extract application on the soluble sugar content of lettuce plants cultivated at three different salinity concentrations. Values are mean of three replicates, and error bars represent ±SD. The bars surmounted with the same letter are significantly comparable (Duncan's multiple-range test) ns, not significant, * significant at $P \leq 0.05$, ** significant at $P \leq 0.01$. S: Salinity, B: Biostimulant application.

Malondialdehyde (MDA) amount increased between 15.93 and 61.22 % at different NaCl levels. However, the biostimulant application decreased the MDA level in lettuce plants. 100 mM NaCl resulted in a maximum reduction in MDA (82.50 %). At 100 mM NaCl, seaweed extract reduced H_2O_2 level by 15.90% compared with the control.

The membrane damage extent was indirectly measured through conductometric measurements of solute leakage from cells. Our findings showed that the amount of electrolyte leakage was considerably elevated when salinity levels were increased (Fig. 7C).

Without the seaweed extract treatment, NaCl-stressed plants experienced a 48.74% increase in electrolyte leakage when compared to the control. The biostimulant treatment partially mitigated this increase. When stimulated plants were exposed to 50 and 100 mM NaCl, the electrolyte leakage level was significantly reduced by 12.47% and 81.71%, respectively, compared to controls.

Antioxidant enzymes activities

The seaweed extract treatment and the NaCl concentration were responsible for the variation in enzyme activities in the present study (Fig. 8).

Significant interaction between salt stress and seaweed extract treatments was noted for all antioxidant enzyme activities, as demonstrated by ANOVA analysis ($p < 0.01$) (Fig. 8).

In untreated plants by biostimulant, Nacl-stress treatment led to an increase in the activity of the antioxidant enzymes CAT (45.24%), SOD (43.37 %) and APX (41.47%), compared with controls.

The highest activity of CAT, APX, and SOD in lettuce leaves was noted in stimulated plants, regardless of the NaCl level.

At 50 mM Nacl, seaweed extract enhanced APX, CAT, and SOD activities by 50.10%, 23.11%, and 29.90%, respectively, while at 100 mM Nacl, only the activity of SOD was increased by 35.46%, paralleled to the control.

Transcript accumulation of genes encoding antioxidant enzymes

Transcript abundance of genes encoding antioxidant enzymes, including *for LsSOD, LsCAT and LsAPX* were quantified in leaf tissue using RT-qPCR (Fig. 9).

Gene expression levels in the leaves of stressed plants by Nacl were higher than those in control plants. The seaweed extract application led to a significant rising in *LsCAT* compared to the controls. However, application of seaweed extract resulted in the most negligible expression of *LsSOD* and *LsAPX* compared to untreated plants. In untreated plants by biostimulant, at 50 mM NaCl, the *LsAPX* gene expressed 4.83 times more in salinity-treated plants than plants treated with nutrient solution (non-stressed controls). At 100 mM NaCl, *LsAPX* was similarly induced in all stressed lettuces and showed an increase of 87.27 % Compared with controls (Fig. 9A).

Expression of *LsAPX* decreased in the stimulated plants by algal extract, still, during severe stress conditions, the leaves of plants treated with biostimulant saw a greater

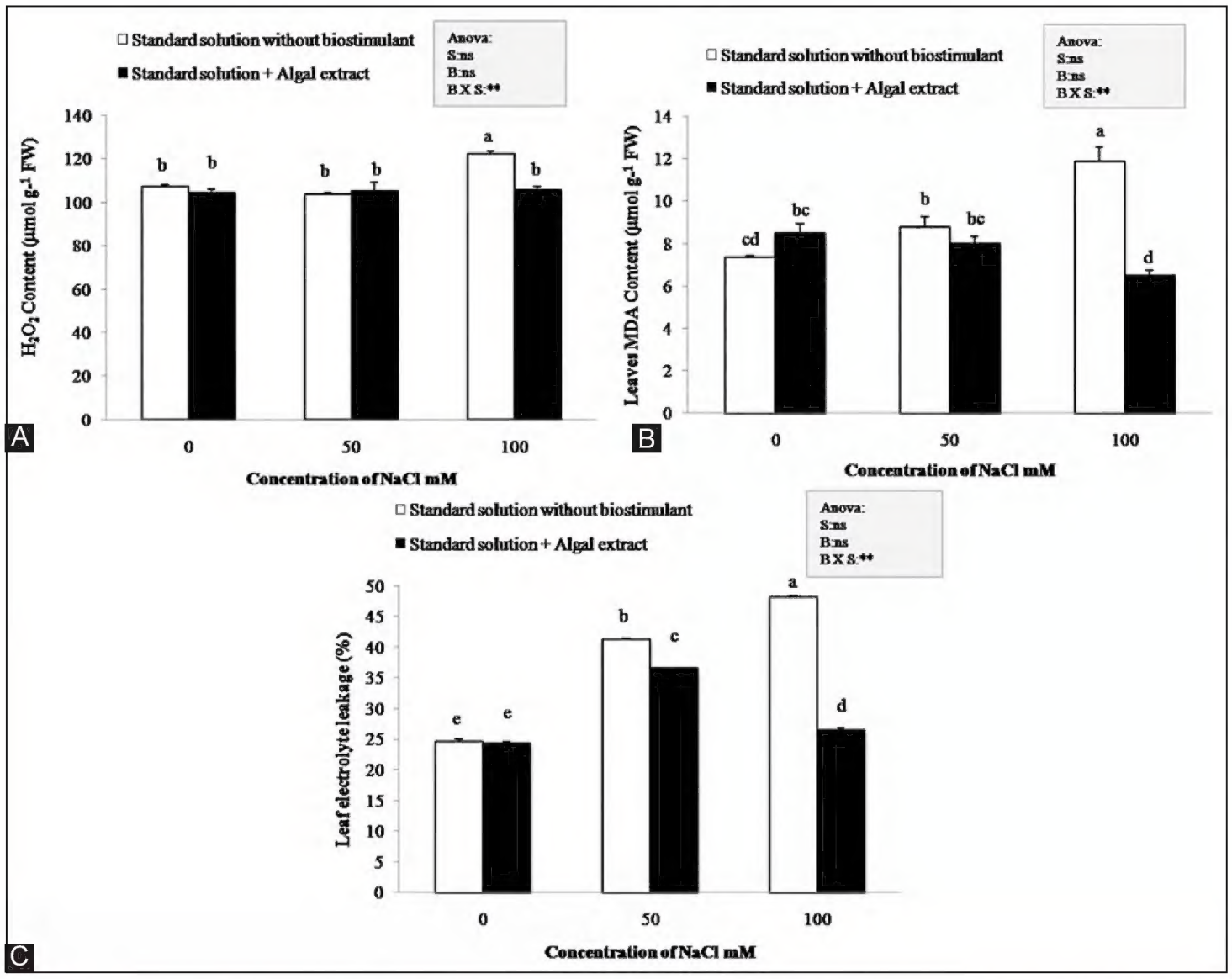

Fig 7. Effects of Algal Extract application on Oxidative Stress indicator Levels (H_2O_2 (A), MDA (B) and Leaf electrolyte leakage (C)) of lettuce plants cultivated at three different salinity concentrations. Values are mean of three replicates, and error bars represent ±SD. The bars surmounted with the same letter are significantly comparable (Duncan's multiple-range test) ns, not significant, * significant at $P \leq 0.05$, ** significant at $P \leq 0.01$. S: Salinity, B: Biostimulant application.

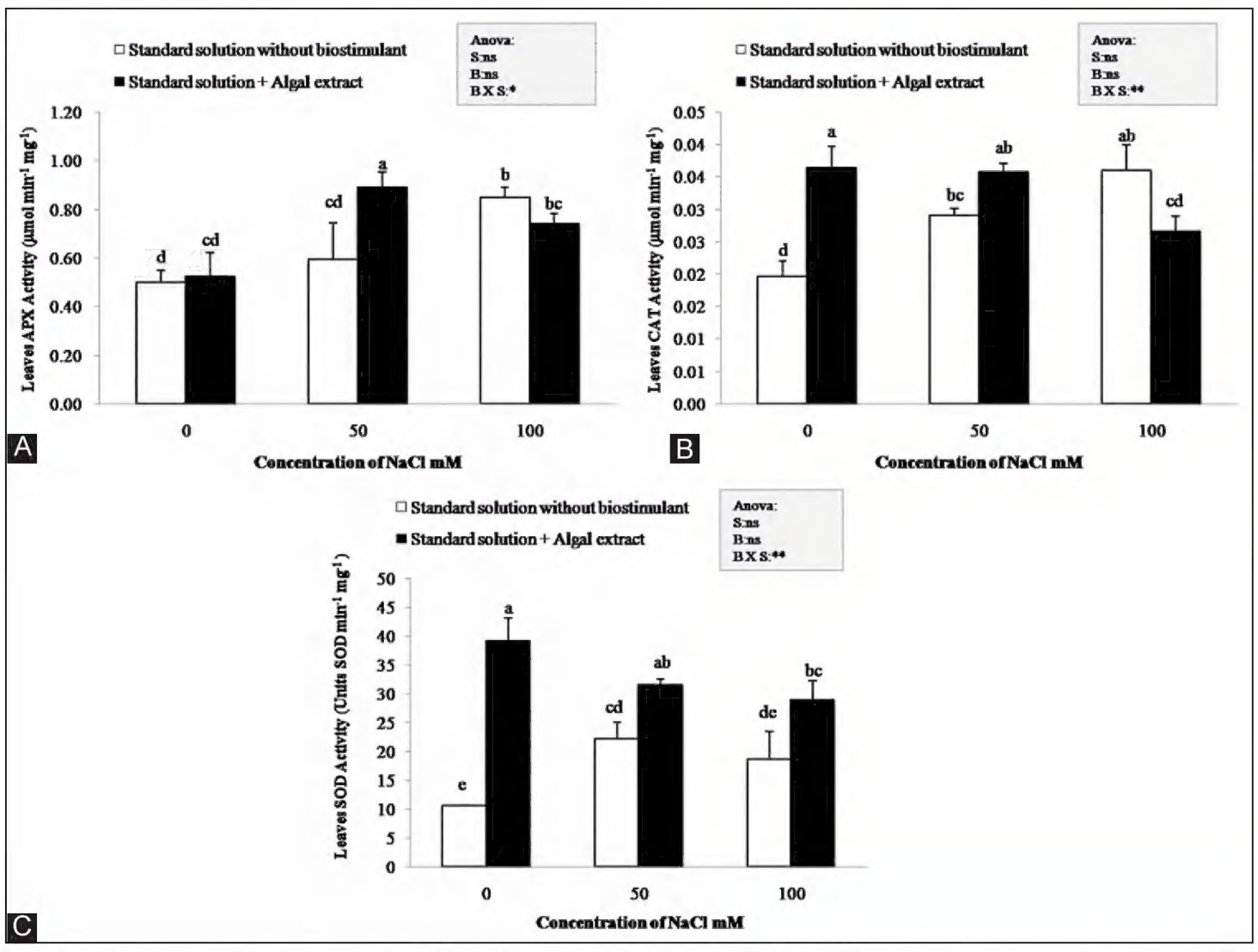

Fig 8. Effects of Algal Extract application on the SOD, CAT (B), and APX (A) activities (C) in lettuce plants cultivated at three different salinity concentrations. Values are mean of three replicates, and error bars represent ±SD. The bars surmounted with the same letter are significantly comparable (Duncan's multiple-range test) ns, not significant, * significant at $P \leq 0.05$, ** significant at $P \leq 0.01$. S: Salinity, B: Biostimulant application.

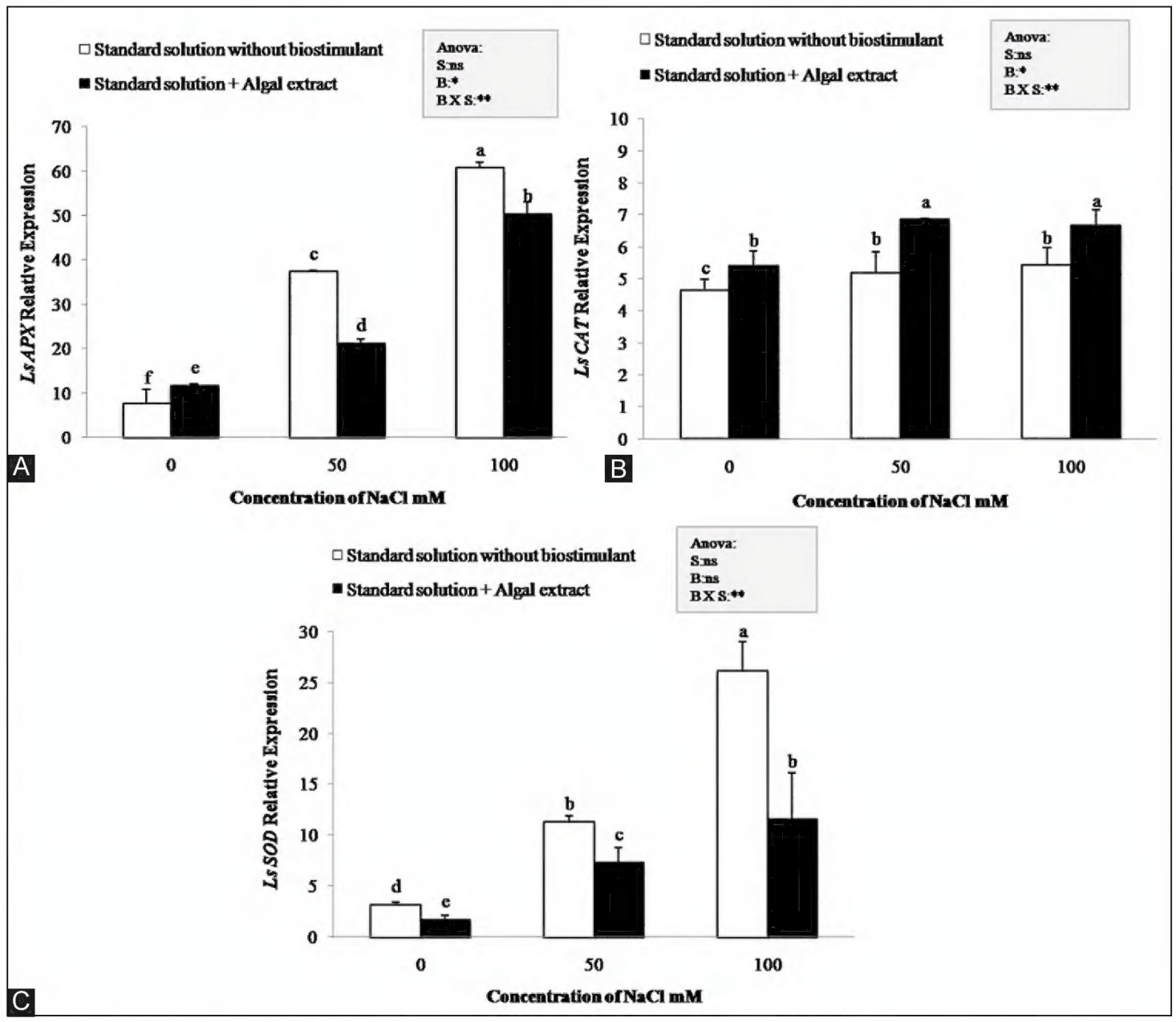

Fig 9. Effects of applying algae extract on the expression of the LsAPX (A), LsCAT (B), and LsSOD (C) genes in lettuce plants cultivated at three different salinity concentrations. Values are mean of three replicates, and error bars represent ±SD. The bars surmounted with the same letter are significantly comparable (Duncan's multiple-range test).

increase in *LsAPX* transcript levels (76.78 %) than in moderate stress conditions (44.77 %) compared with controls (0 mM NaCl) (Fig. 9A).

The biostimulant non-treated and treated plants experienced an upregulation of *LsCAT* due to salt challenge. *LsCAT* expression was maximum in stressed and treated plants by biostimulant. *LsCAT* showed a higher increase at 100 mM NaCl in the leaves of the treated plant by biostimulant (18.86 %) than in untreated plant by biostimulant (5.06 %) compared with control plants (0 mM NaCl) (Fig. 9B).

The levels of *LsSOD* in both treated and untreated lettuces by seaweed extract, increased similary under the effect of moderate and severe salt stress. Untreated plants with seaweed extract at 100 mMNaCl (87.71%) displayed a greater increase in *LsSOD* upregulation compared to controls (Fig. 9C).

Correlation analysis

Specific interactions between growth, physiological traits, leaf tissue integrity, and enzymatic status were determined based on biostimulant treatment (control, seaweed extract) (Fig. 10).

Under control conditions, correlation analysis revealed a significant positive relationship ($p< 0.01$) between Osmoprotectant Contents (proline and sugars) and levels of oxidative stress indicator (H_2O_2, MDA and electrolyte leakage). In terms of enzyme activities, Sodium content and electrolyte leakage showed a positive correlation with CAT, SOD and APX activities. Comparison of transcript accumulation with respective enzyme activities confirmed the existence of a relationship between *LsAPX*, and *LScat* ($r=0.96$; $p< 0.001$) expression and APX and CAT activities ($r=0.81$; $p< 0.01$).

Under biostimulant conditions, a significant positive relationship ($p< 0.01$) was obtained between osmoprotectant contents (proline and sugars) and sodium content. SOD activity was correlated with MDA, DW, P, TLA, Fv/Fm and RWC; while APX was positively correlated with sodium amount ($r=0.8$; $p< 0.05$), osmoprotectant contents (proline (($r=0.67$; $p< 0.05$) and sugars ($r=0.88$; $p< 0.01$)) and *LsCAT* (($r=0.92$; $p< 0.001$). CAT activity was linked with SPAD and TLA. Sodium accumulation was negatively correlated with photosynthetic activity (Fv/Fm) and with growth parameters (TLA and DW), MDA, SOD activity and its gene expression (*LsSOD*). Correlation of transcript

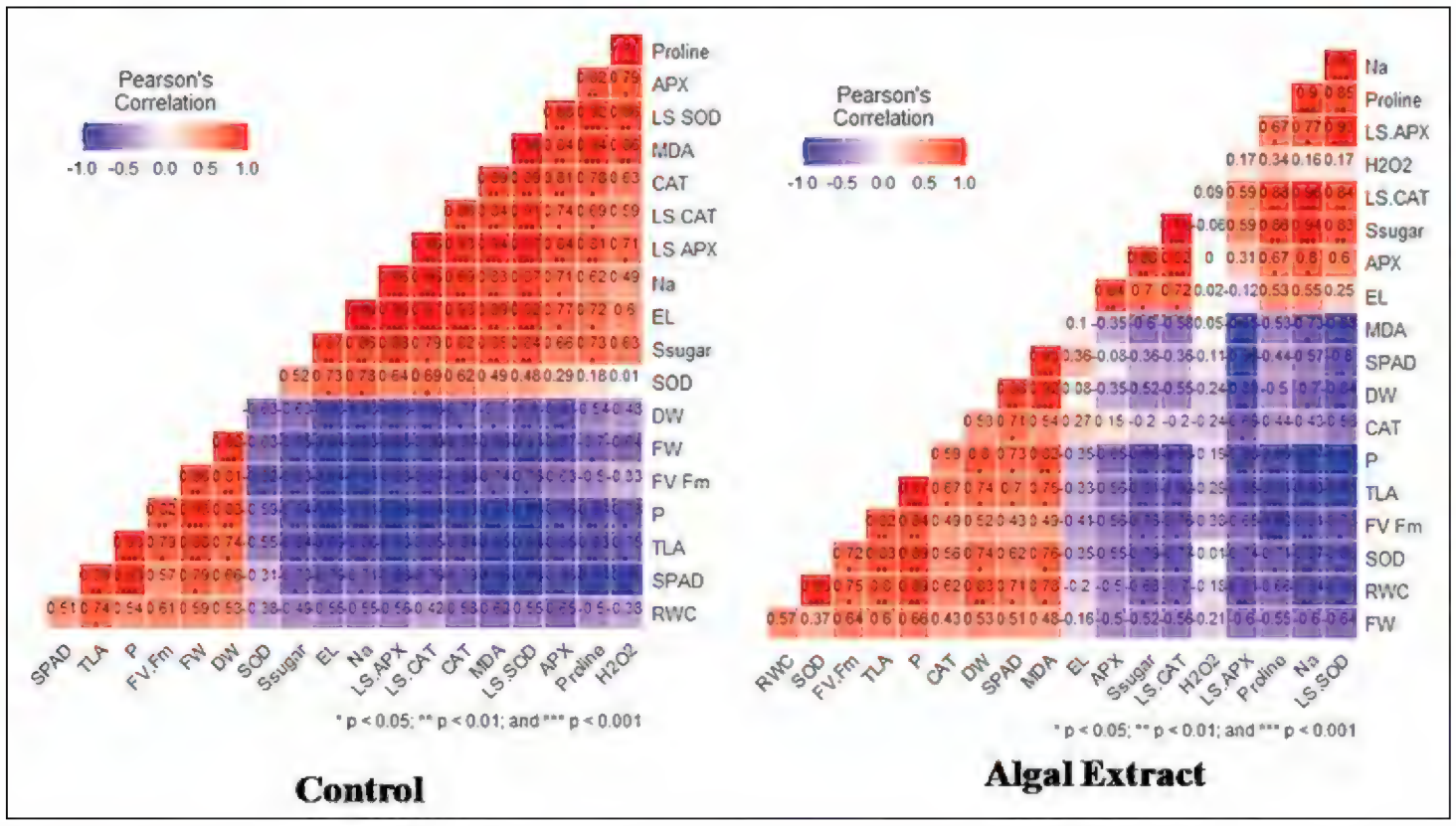

Fig 10. Pearson correlation analysis between, physiological traits, leaf tissue integrity and enzymatic status following Algal Extract application. Rectangles in red and blue indicate positive and negative correlations, in that order. Three replicates were used to get the r values. *, **, ***Significant at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$, respectively.

accumulation with respective enzyme activities showed No relationship between *LsAPX*, *LsSOD* and *LScat* expression and APX, CAT and SOD activities.

DISCUSSION

Algae are capable of colonizing even complex habitats as autotrophic photosynthetic organisms. Seaweeds are subjected to continuous, occasionally extreme variations in water salinity, temperature range, brightness of light, and nutrition accessibility. As a result, algae acquired the capacity to produce a wide range of secondary compounds. Those are necessary to respond to and adjust to abiotic stress promptly. According to various studies AEs stimulate plant development by means of a variety of metabolites and receptor molecules, including, phytohormones, carbohydrates, amino acids, tetraterpenenoids, vitamins, and polyamines (De Morais et al., 2015).

The findings of Gómez-Guzmán et al. (2018), which demonstrated that flavonoids are among the most prevalent secondary metabolites in green algae, are consistent with our own.

The identification of phenolic compounds from algal extract in our study suggests that these algae may possess significant antioxidant activities. Morai et al. (2020), showed in this context that the development of lettuce seedlings exposed to salinity was significantly increased via seaweed *Ulva lactuca* extract. This increase can be explained by the existence of several biomolecules including phenolic compounds, in this green algae extract.

It is well known that marine algae have a high phenolic content. These chemical compounds have a tendency to concentrate under stress and can chelate metal ions, neutralize reactive oxygen species (ROS), and stabilize membrane proteins (Huang et al., 2014).

Salinity as a stressor affects several plant metabolic pathways by causing an overabundance of ROS, which adversely affects plant performance and yields (Rady & Hemida, 2016). Plants under stress activate several particular defensive mechanisms, such as ion hemostasis, osmoregulation, and enzymatic and non-enzymatic antioxidant production, to prevent salt toxicity. Nevertheless, the defensive systems of plants are not enough to protect themselves from prolonged stress. Therefore, exogenous applications must support plants (Rady et al., 2021). The algal extract was recently utilized as an extremely efficient multi-action biostimulant (Semida et al., 2019).

In the current study, adding salt negatively affected fresh lettuce yield, dry weight and leaf area proving that lettuce mainly exhibits an osmotic stress response. The development of numerous plants, such as *Lactuca sativa* L., have been demonstrated to be negatively impacted by salinity (Shin et al., 2020). The impact of salt on plant development and productivity has been attributed to a decrease in leaf surface. This decrease affects photosynthesis, water, and mineral uptake (Shannon and Grieve, 1998). The increased concentration of Na^+ and Cl^- ions, as well as disrupted mineral supplies, may result in a reduction of morphological traits (Ashraf and Harris, 2013). The general decrease in plant biomass may be linked to the inhibition of photosynthesis. This might be caused

by the inhibition of mesophyll conductance and stomatal restrictions (Chaves et al. 2009).

On the other hand, when algal extract was administered to lettuce plants following NaCl treatment, the extent of growth decline was considerably mitigated in comparison to non-treated plants. The active components of the algal extract that stimulate growth may explain the increase in plant growth traits following treatment.

According to reports, algae has macro- and micro-elements, vitamins, auxins, cytokinins and abscisic acid (Khan et al., 2009),these can affect cells' biological activities and reduce salinity's destructive impact on seedling growth. Hemida et al. (2014) claim that the positive effects of Ulva extract on plants under saline stress can be attributed to the abundance of biochemical substances such as glutathione, proline, and ascorbic acid. These compounds are necessary to induce antioxidant systems that reduce the concentration of reactive oxygen species in the plant. Ibrahim et al. (2014) discovered that Ulva lactuca extract significantly increased the development of wheat seedlings in salinity-stressed conditions.

According to this investigation, the concentrations of Sodium (Na^+) and Potassium (K^+) in leaf tissues suggest that lettuce has entered a stage where it is specifically affected by salt. When the amount of NaCl in growth medium was increased from 50 to 100 mM, it led to an increase in the uptake and accumulation of Na, causing nutritional disorders in the lettuce. Several mechanisms have been implicated in the nutritional imbalance of plants, including and the osmotic effects of salts the competition between both Na^+ and K^+ assimilation in roots (Munns, 2005). Consequently, plants are more susceptible to particular ion damage and mineral deficiencies, leading to stunted growth and productivity loss. According to Marschner (2012), nutrient assimilation is crucial for maintaining homeostasis and plant growth, especially in the face of edaphic challenges.

In the current study, the application of algal extract resulted in a decrease in plant sodium uptake and an improvement in plant potassium uptake under salt stress, as compared to untreated plants. The substantial potassium accumulation and the lowest sodium levels in the lettuce plants treated with biostimulant may have maintained the osmotic potential of their cells, preventing cell sodium accumulation at toxic concentrations. The effects of applying biostimulants on agronomical parameters need to be considered at both physiological and biochemical measurements. In this study, the main physiological changes observed in lettuce leaves treated with biostimulants were fluorescence and chlorophyll concentration in the leaf.

The untreated lettuce plants showed a significant reduction in the SPAD index, indicating low chlorophyll level, under salt-stress conditions. This implies that, as reported by Rouphael et al. (2015), chlorophyll degradation occurred, most likely as a result of ROS's detrimental effects on chloroplasts. However the plants treated with biostimulant were able to retain a greater SPAD index regardless of nutrient solution treatments. Algal extract's beneficial effect on protecting chlorophyll from degradation may be due to better uptake of divalent cations notably magnesium cation (Mg^{2+}) and metallic cation ($Fe2^+$), which are necessary for chlorophyll production(Sheng et al., 2008). Several authors including Colla et al. (2008), Ertani et al. (2013), and Rouphael et al. (2010) have demonstrated that the application of plant biostimulants can help restore acceptable amounts of chlorophyll content under salinity. Our own findings support these results.

The Fv/Fm ratio showed that the maximal quantum yield of photosystem (PS) II decreased along with the decline in the SPAD index, a measure of plant chlorophyll content, in lettuce plants that were not treated. This shows that PSII's electron transport was inhibited by salinity, leading to photoinhibition. Stress and injury to the PSII may be the cause of this(Demmig-Adams and Adams, 1992). According to the findings of Shu et al. (2013) and Cai et al. (2014), salt stress can cause a decrease in Fv/Fm. This is mainly due to a blockage of the transmission of electrons at the PSII reaction center's acceptor side.

Higher salinity can result in decreased photosynthesis due to reduced stomatal activity, suppression of carbon-absorbing processes, diminished photochemical efficiency (Dubey, 1997).

By foliar spraying lettuce plants with algal extract, the photosynthetic efficiency was typically increased under normal and salt conditions, compared to the matched controls.

Maintaining high Fv/Fm levels in lettuce plants through foliar spraying with algal extract may delay photoinhibition and improve photosynthesis, leading to increased lettuce yield.

The osmotic effect of salinity most likely causes growth inhibition due to reduced water uptake, since cellular growth is primarily facilitated by preserving cellular water reserves under salt conditions (Negrao et al., 2017).

Based on the results of our experiment, we observed that he RWC of lettuce plants decreased under salinity, however, we also found that the application of algal extract led to an increase in RWC. One important strategy for preserving optimal plant growth in salt conditions is to

maintain a considerable amount of relative water in the leaves (Siddiqui et al., 2014).

According to Marcek et al. (2019), active metabolic reprogramming of plants for osmotic stress, allowing the plants to maintain regular cell processes and development, was identified by increased RWC, improved photosynthetic activity, more open stomata, and a greater transpiration rate.

To achieve the equilibrium of cellular water, osmotic regulation which involves an accumulation of soluble proline and sugars is considerably more successful in plants that tolerate salinity. The production of osmolytes, such as proline, is essential for reducing ROS and preventing injury to cell membranes and proteins (Kaur and Asthir, 2015).

The accumulation of free proline acts as one of the non-enzymatic antioxidant processes. Furthermore, Rosa et al. (2009) found that the main class of organic solutes that are appropriate for plants, soluble sugars, considerably reduces environmental stress through osmotic modification, which is a vital process for plants to respond to a variety of stress factors. According to Pattangul and Thitisaksakul (2008), Elevated levels of sugar inside the cell's cytoplasm may lead to subsequent adverse that restrict carbon dioxide uptake.

Reactive oxygen species were produced more quickly due to lower photosystem activity, electron transfer rates, and limited CO_2 fixation. This implies that electrons are moving toward oxygen molecules instead of assimilating carbon (Zribi et al., 2008).

The plants treated with algal extract exhibited the highest levels of proline and soluble sugar synthesis in our investigation, which may have improved osmotic regulation and enhanced cell defense against salt stress.

Plant acclimation to salt conditions is frequently related with a rises in ROS production, such as hydrogen peroxide (H_2O_2), hydroxyl radical (HO^-), and singlet oxygen (O_2), which are occasionally harmful to cells. According to Ahmad et al. (2012), the disturbance of the system that transports electrons and metabolic activities that are occurring in chloroplasts, mitochondria, and peroxisomes generates an increase in the formation of ROS under stressful conditions. ROS can be effectively eliminated under normal circumstances by both enzymatic and non-enzymatic antioxidant systems. On the other hand, during salt stress, an immune system imbalance may result from the overproduction of ROS (Sofo et al., 2015). One of the main oxidizing species, H_2O_2, can have a detrimental effect on cellular function if it accumulates in extremely excessive quantities (Lee et al., 2013). Furthermore, plants acquire Na^+ in a nonselective manner as a result of H_2O_2 generation in salinity. In spite of this, the biostimulant greatly decreased H_2O_2 in comparison to lettuce samples that had been raised just with Salt.

In light of this last point, the other measurement examined, in this study, namely the amount of malondialdehyde (MDA), should be investigated. Our study's findings show that when lettuce was treated with sodium chloride only salt dramatically raised the quantity of MDA; however, when the plants were biostimulated, the amount of MDA decreased. MDA content increases in situations of extreme oxidative stress and antioxidant defenses cannot modulate ROS (Liang et al., 2018). The results of this investigation demonstrate that algal extracts can enhance the oxidative state of lettuce and align with previous research findings regarding beneficial influence of biostimulants (Panfili et al., 2019; Paradikovic et al., 2019).

ROS can cause alteration to cell membrane lipids, proteins, and nucleic acids, leading to electrolyte leakage and senescence. The membrane of the plasma is the initial location for ion-specific saline damage. Therefore, Electrolyte leakage content is regarded as a key indicator of plant resistance (Ashraf and Ali, 2008).

The increase in NaCl levels increased electrolyte leakage, indicating that increased salt causes worse destruction of cell membranes. However, using algal extract resulted in less electrolyte leakage, which likely led to less membrane damage.

Haghighi and Pessarakli (2013) and Tuna et al. (2008) observed that silicon decreased electrolyte leakage in salt-stressed tomatoes and wheat when comparing plants that were treated with silicon and did not receive treatment. Furthermore, inoculation of plant-growth-promoting rhizobacteria (PGPR) decreased cell membrane damage in cucumber plants under salt stress (Kang et al., 2014) and maize (Marulanda et al., 2010), compared to the uninoculated control.

Plants employ multiple mechanisms, including enzymatic and non-enzymatic antioxidants, for eliminating reactive oxygen species (ROS) in their reaction to stresses. To mitigate damages caused by (ROS), plants have evolved a sophisticated antioxidant system. Superoxide dismutase (SOD) neutralizes O2 and generates H_2O_2, whereas catalase (CAT) and ascorbate peroxidase (APX) are needed for H_2O_2 elimination (M'Hamdi et al., 2009).

The increase in SOD, CAT, and APX antioxidant enzyme activity in lettuce plants treated with algal extract coincided with an increase in plant antioxidants under both salt-free and moderate salt conditions. Overall, our findings indicate

that algal extracts activate antioxidant enzyme activities and prevent oxidative damage in lettuce plants. Additionally, the upward regulation of enzyme activity resulting from the use of algal extract may be explained by the biocompounds, soluble sugar, polyphenol, and proteins, found in this extract. Antioxidant enzymes may be supported by these substances (Ben Mrid et al., 2018).

According to the rise in antioxidant activity of enzymes, algal extract induced an effective ROS scavenging mechanism that protected lettuce plants from extensive oxidative damage.

Understanding the antioxidant responses to salinity at the transcriptomic level is crucial to propose novel approaches for improving tolerances to environmental stress. Various antioxidant gene copies may be variably modulated under harsh climatic conditions (Ara et al., 2013).

Similarly to the accumulation of antioxidant enzymes, the transcription of the corresponding SOD, CAT, and APX genes was found to be stimulated in treated lettuce plants under salt conditions.This finding suggests that improved transcription of the SOD, CAT, and APX genes could increase the activity of the SOD, CAT, and APX enzymes, improving cellular defense against oxidative stress imposed by high salt (Hu et al., 2012). In support of our finding, several studies have demonstrated the overexpression of genes encoding antioxidant enzymes during stress, both with and without the use of biostimulants. Hernandez et al. (2000), found that a NaCl-tolerant pea variety had higher levels of SOD and APX gene expression than the sensitive variety. Other plants exposed to salt, such as *Lotus japonicus* (Rubio et al., 2009), *Limonium sinense* (Zhang et al., 2014), and tomato (Aydin et al., 2014), have also been shown to have promoted SOD gene expression.

In our study, the expression level of *LsCAT* was considerably enhanced by supplementing algal extract to NaCl-treated plants. This up-regulation triggered increased CAT activity, as determined in biochemical analyses in both treated and untreated plants by the algal extract.

In a different study, Gondim and collaborators (2012) investigated the impact of pretreatment with H_2O_2 leaf spray on plant development. The results of the experiment showed that antioxidant enzyme activity was enhanced by H_2O_2 spraying, and CAT was the most reacting enzyme to H_2O_2.

Elevated CAT activities seem to be associated with the regulation of gene expression. Plants with higher CAT activity displayed lower oxidative damage, indicating the enzyme's defensive function.

The current research validates the overall association between photosynthesis ability and leaf area, supported by the theory of Reich et al. (1999) that none of the species can expand photosynthetic potential without rising leaf area because of biophysical restrictions. Salt overload has an impact on a number of plant biochemical processes, including pigment production and photosynthesis (Colla et al., 2010).

In control conditions, Na accumulation was positively correlated with MDA, Electrolyte leakage, soluble sugars, APX, CAT, *SOD*, *LsSOD*, *LsCAT*, and *LsAPX* and it was negatively correlated with potassium content, growth and physiological parameters.

An overabundance of sodium ions in the cytosol has detrimental effects on cellular membranes, which ultimately lower physiological and biochemical activities by limiting cytosolic metabolic processes and causing the escape of electrolytes (Ramadan et al., 2019). The accumulation of higher levels of MDA contents in salt-stressed leaves led to an increase in electrolyte leakage, this could be the result of destroying membranes caused by ROS-induced oxidative injury.

Higher levels of salt and extended exposition to sodium chloride stress cause oxidative stress in plants (Rasool et al., 2013). Sodium chloride and osmotic stressors stimulate the generation of ROS that cause oxidative injury in plants (Abdel Latef and Chaoxing, 2014). To cope with the negative effects, plants have an adequately controlled antioxidant mechanism that preserves biological molecules from additional damage caused by oxidative stress (Rasool et al., 2013).

SOD, CAT, APX, and glutathione reductase (GR) are the ROS-removing enzymes (Evelin and Kapoor, 2014). These enzymes are distributed in different Cellular organelles as isoenzymes, especially in mitochondria and chloroplasts (Apel and Hirt, 2004). According to Abdel Latef and coauthor (2014), accumulating osmolytes, especially soluble sugars, is another strategy for overcoming the osmotic shock brought on by salt.

The expression profile of the *LsCAT* and *LsAPX* genes and their enzyme activities have a strong positive correlation, this does not apply to the LsSOD gene and its matching enzymes. The correlation found among the RNA quantities and the CAT and APX enzyme activities emphasizes the essential function these enzymes play in regulating ROS under situations of salt stress as well as their implication in salt tolerance mechanisms. The non-existence of an association between SOD RNAm abundance and the activity of enzymes may be caused by the complicated

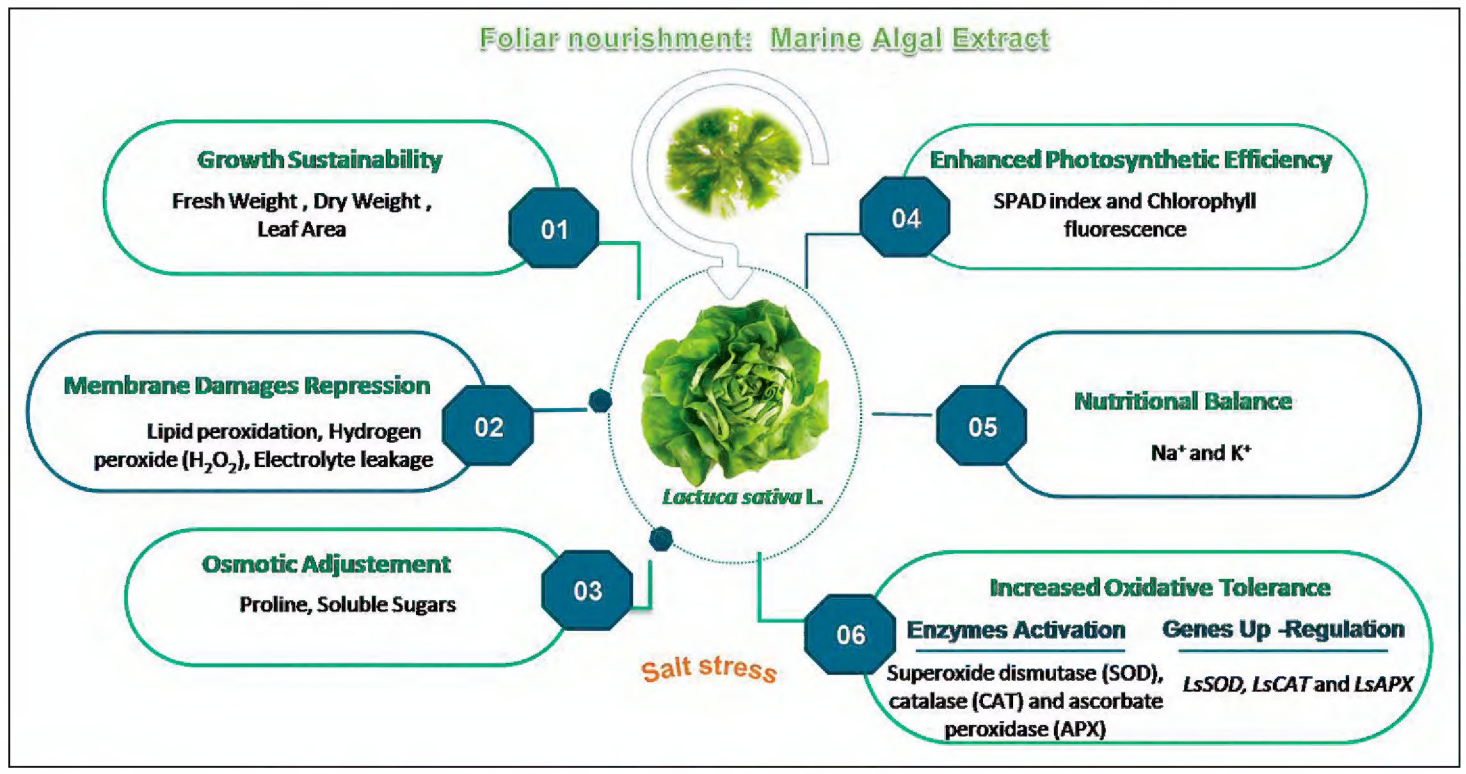

Fig 11. A diagram depicting the growth modulation in lettuce plants by Algal Extract application involving hysiological processes, osmotic adjustment, antioxidant gene regulation, and enzymatic activity under salt conditions.

processes regulating gene expression and the day-to-day fluctuations in the expression of genes linked to the oxidative stress responding (Adem et al., 2014).

Under saline stress, plant metabolism has been improved due to the protective impacts of seaweed extract on different tolerance systems. It appears that improved photosynthesis mechanism performance seems to be responsible for the lower decline in lettuce growth was observed in plants that received the biostimulant. The findings show a significant association between the osmolytes (proline) and the accumulated amounts of sodium. Besides, Algal extract improves antioxidant potential as well as offers plants extra antioxidant properties to combat salt-stressing effects. By foliar spraying plant leaves, the antioxidants in *Ulva* extract allowed the plant to respond to challenges in an adaptive way that reflected the growth and development of the plant (Fig. 11).

CONCLUSION

According to this investigation, the application of an exogenous algal extract can effectively reduce the detrimental effects of salinity stress on the development and physiological characteristics of lettuce plants.

This is achieved by increasing the levels of potassium, compatible solutes, enzymatic properties, and gene expressions while reducing the levels of sodium, oxidative stress indicators, and membrane damage associated with oxidative stress.

This study additionally demonstrates the induced beneficial function of LsCAT toward oxidative damage by biostimulant application. These favorable findings are attributed to the bioactive elements (e.g., sugars, proline) of seaweed extract, playing a key part in plant growth and metabolic adaptation to salinity stress.

However, improved yields and product quality can be achieved by using Marine Algal Extract extract in other crops, whether under normal or stressful conditions.

To fully understand how this interesting seaweed extract stimulates signaling processes and physiological reactions to abiotic stress, more investigation is needed.

Conflicts of interest

The authors declare no conflict of interest.

Authors' contributions

Dhouha Aloui carried out experiments, analyzed experimental results and drafted the manuscript. Fatma Kalleli designed the experiment, analyzed experimental results, performed the statistical analysis and wrote the manuscript. Ghassen Abid carried out the biochemical and molecular analysis and assisted wrote the manuscript; Chahine Karmous assisted with the statistical analysis and contributed to the interpretation of the results. Mariem Manaa assisted the greenhouse and laboratorial analysis, Mahmoud M'Hamdi was responsible for designing the experiment, supervising the assays, and revising the manuscript.

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